

# Convergence of Specific Immunoglobulin E Detection Methods: From Extract-Based Tests to Molecular Allergy Diagnostics

## ZBIEŻNOŚĆ METOD WYKRYWANIA SWOISTEJ IMMUNOGLOBULINY E: OD TESTÓW OPARTYCH NA EKSTRAKTACH DO DIAGNOSTYKI MOLEKULARNEJ ALERGII

ALEKSANDRA KUBISIAK<sup>1</sup>, JAROSŁAW MIJAS<sup>1</sup>, ZENON BRZOZA<sup>2</sup>

1. Clinical Department of Pediatrics of the Hospital in Strzelce Opolskie, Institute of Medical Sciences, University of Opole  
2. Department of Internal Diseases, Allergology, Endocrinology and Gastroenterology, Institute of Medical Sciences, University of Opole

\* Autor korespondencyjny

### Streszczenie

**Wprowadzenie:** Choroby alergiczne stanowią istotny i narastający problem zdrowotny. Ich prawidłowa diagnostyka wymaga połączenia dokładnego wywiadu klinicznego z odpowiednio dobranymi metodami *in vivo* i *in vitro*. W ostatnich latach klasyczne metody diagnostyczne zostały uzupełnione o nowoczesną diagnostykę molekularną.  
**Cel pracy:** Celem pracy było porównanie konwencjonalnych i nowoczesnych metod diagnostyki alergii IgE-zależnej oraz ocena ich przydatności klinicznej.

**Materiał i metody:** Przeprowadzono przegląd piśmiennictwa dotyczącego diagnostyki alergii, obejmujący testy skórne, oznaczanie swoistych IgE z użyciem ekstraktów alergenowych, diagnostykę komponentową oraz testy multipleksowe i point-of-care. Analizie poddano publikacje z baz PubMed i Google Scholar oraz literaturę specjalistyczną.

**Wyniki:** Testy skórne punktowe pozostają wartościowym narzędziem pierwszego rzutu, wykazując wysoką zgodność z oznaczeniami swoistych IgE. Metody *in vitro* oparte na ekstraktach są łatwo dostępne, lecz obciążone ryzykiem reakcji krzyżowych i wyników fałszywie dodatnich, m.in. z powodu CCD. Diagnostyka molekularna umożliwia dokładne różnicowanie uczulenia pierwotnego od reakcji krzyżowych, ocenę ryzyka anafilaksji oraz właściwą kwalifikację do immunoterapii.

**Wnioski:** Diagnostyka alergii powinna mieć charakter etapowy i zintegrowany. Metody konwencjonalne i molekularne są komplementarne i powinny być stosowane łącznie, zawsze w odniesieniu do obrazu klinicznego pacjenta.

**Słowa kluczowe:** alergia IgE-zależna; diagnostyka molekularna; diagnostyka oparta na komponentach alergenowych (CRD)

### Summary

**Introduction:** Allergic diseases represent a significant and growing health problem. Accurate diagnosis requires the integration of a detailed clinical history with appropriately selected *in vivo* and *in vitro* diagnostic methods. In recent years, classical diagnostic approaches have been complemented by modern molecular allergy diagnostics.

**Aim:** The aim of this study was to compare conventional and modern diagnostic methods for IgE-mediated allergy and to assess their clinical usefulness.

**Material and Methods:** A review of the literature on allergy diagnostics was conducted, including skin tests, determination of allergen-specific IgE using allergen extracts, component-resolved diagnostics, and multiplex and point-of-care assays. Publications from the PubMed and Google Scholar databases, as well as specialist literature, were analyzed.

**Results:** Skin prick tests remain a valuable first-line diagnostic tool and show high concordance with measurements of allergen-specific IgE. *In vitro* methods based on allergen extracts are widely available but are limited by cross-reactivity and the risk of false-positive results, including those related to cross-reactive carbohydrate determinants (CCD). Molecular diagnostics enable precise differentiation between primary sensitization and cross-reactivity, assessment of anaphylaxis risk, and appropriate qualification for allergen immunotherapy.

**Conclusions:** Allergy diagnostics should follow a stepwise and integrated approach. Conventional and molecular methods are complementary and should be used together, always interpreted in the context of the patient's clinical presentation.

**Key words:** IgE-mediated allergy; molecular diagnostics; component-resolved diagnostics

### Adres do korespondencji/Address for correspondence

Aleksandra Kubisiak

E-mail: aleksandrakubisiak1@gmail.com

tel.: 724846094

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## Introduction

In 1905, the Viennese paediatrician von Pirquet introduced the concept of allergy to medicine as a different immune reactivity of the body. According to the classical definition, an allergy is a specific adverse reaction dependent on an immune response secondary to contact with a foreign antigen. Allergic diseases are an increasingly common problem in modern medicine. [1]

Allergic symptoms can affect any organ in the human body, but are most often concentrated in the upper and lower respiratory tract, skin, eyes and can involve many different systems simultaneously (i. e. anaphylaxis). [2]

When assessing a patient suspected of allergy, we should look broadly at the patient; correctly asked questions during the physical examination and a thorough physical examination of the patient greatly facilitate the decision to perform further laboratory tests and bring the patient closer to a correct diagnosis.

On a daily basis, doctors use two diagnostic methods to diagnose allergy; *in vitro* tests and *in vivo* tests. It is important to remember that the results of these tests are only relevant in correlation with the patient's clinical symptoms. *In vivo* tests are skin tests - prick, intradermal and epidermal; they differ in the method, yet they are a standardised tool for checking the skin reaction to a given allergen. The *in vivo* method is particularly valuable for distinguishing allergies from pseudoallergic reactions. Numerous cross-sectional studies, based on extensive analyses of medical records and comparisons of various diagnostic modalities, consistently demonstrate that skin-prick testing (SPT) constitutes a valuable and recommended first-line diagnostic tool for assessing patients with suspected inhalant allergy. As shown by Szmyd et al. [3] there is a strong positive correlation between SPT results and serum concentrations of specific IgE (sIgE). The authors emphasize that combining data derived from these two methods enables accurate identification of inhalant allergy in approximately 95% of cases, underscoring their high concordance and complementary diagnostic value. Similar conclusions were drawn in a large retrospective analysis of 1,711 patients from central Poland conducted by Majkowska-Wojciechowska et al. [4]. The investigators demonstrated that SPT not only correlates well with sIgE results but also shows high agreement with provocation tests - including nasal, conjunctival, and other challenge procedures - which represent the gold standard in the clinical assessment of hypersensitivity to airborne allergens. These observations confirm the utility of SPT as a rapid, sensitive, and cost-effective diagnostic tool. Despite their widespread use, the aforementioned tests have a number of drawbacks. The main limitations include the potential subjectivity in the interpretation of results by the person performing the test, the lack of standardised extracts causing variability between tests, the inability to assess antigenic recombinants and, very rarely, the elicitation of a systemic reaction to a particular allergen. Limitations due to certain skin diseases and the use and inability of patients undergoing skin testing to discontinue antihistamines should also be borne in mind. [5] *In vivo* methods also include provocation trials with sensitising substances. [6]

These limitations of *in vivo* methods contribute to a significant increase in the role of *in vitro* tests in the

diagnostics of allergic conditions - determination of allergen-specific IgE and total IgE concentration.

Before analysing the diagnostic possibilities of detecting specific IgE antibodies, let us recall in a few words what these antibodies are.

### The aim

IgE antibodies are specialised proteins, a type of antibody that plays a key role in the body's immune response, being one of the five main groups of immunoglobulins (IgA, IgM, IgG, IgD, IgE). They are produced by the immune system after contact with a specific allergen. Compared to other classes, IgE glycoproteins make up a small percentage of all immunoglobulins in the body, but are extremely important in defence against parasites and in the pathogenesis of allergic diseases. [6,7]

IgE antibodies are glycoproteins with a structure of four polypeptide chains: two epsilon heavy chains and two light chains (kappa or lambda). Each IgE antibody has a specific structure that allows it to bind to a specific antigen. Their unique structure allows high affinity for the FcεRI receptors (activation of these receptors by antigen-bound IgE is crucial for mast cell degranulation), which are present on the surface of mast cells and basophils.

Immunoglobulins E are produced by B lymphocytes following their activation by antigen-presenting cells, such as macrophages and dendritic cells, in the presence of signals from type 2 (Th2) helper T cells. The secretion of interleukins (IL-4, IL-13) by Th2 is central to the classic change from immunoglobulin classes to IgE. [7]

### Mechanism of IgE action in allergic reaction.

1. Sensitisation: The first contact of the organism with an allergen leads to the presentation of the antigen by dendritic cells and macrophages and is recognised by helper T lymphocytes (Th2). Activated Th2 lymphocytes secrete cytokines such as IL-4 and IL-13, which stimulate B lymphocytes to change antibody classes and produce IgE. These antibodies bind to FcεRI receptors on the surface of mast cells and basophils, 'sensitising' these cells to future contact with the allergen.
2. Re-contact and activation: Subsequent exposure to the same allergen results in binding of the allergen to IgE attached to receptors on mast cells or basophils. This bond leads to the so-called cross connection of FCER1 receptors. This triggers a number of intracellular signalling pathways, including the activation of tyrosine kinases (e.g. Lyn, Syk) which cause cell degranulation.
3. Releasing mediators: Degranulation of mast cells and basophils leads to the immediate release of histamine, leukotrienes, cytokines and other compounds that trigger the symptoms of an acute allergic reaction.

## Allergen-specific IgE determination

Nowadays, we have the possibility to detect the concentration of specific IgE by a wide range of laboratory diagnostic methods. Conventional approaches to allergy diagnostics, in addition to PTS, include assessment of allergen-specific IgE in the blood, using only allergen extracts for testing. [7,8]

An antigenic extract is a mixture of allergenic and non-allergenic proteins from a specific allergenic source. Extracts may contain many non-allergenic molecules and may differ in the number of allergenic proteins and their composition. Extracts from natural sources, mainly used in traditional serological diagnostics, are increasingly being replaced by antigenic molecules. By antigenic molecule we mean a single protein or rarely, a carbohydrate grouping that can trigger an allergic immune response in the body - it can be naturally derived so-called native molecule or genetically engineered so-called recombinant molecule. [10]

Wide et al. in 1967 first described a radioimmunoabsorption method used for the precise determination of specific immunoglobulin E directed against a particular antigen. Over a number of years, methods of allergy diagnosis have been greatly improved. Radioimmunoabsorption introduced by Wide has gradually been superseded by an enzymatic method using enzyme-labelled monoclonal antibodies. Later, a technique using antigen binding on solid and liquid substrates was introduced. [11].

Currently, immunoenzymatic methods are most commonly used in laboratory practice for the determination of sIgE; they can be divided into quantitative, semi-quantitative and qualitative methods. With these methods, we are able to determine the concentration of antigen-specific IgE antibody for a specific antigen - single-parameter tests (singleplex) or simultaneously assess the concentration of specific IgE for multiple allergens – multiparameter tests (multiplex).

The quantitative method accurately determines IgE levels expressed in international units of IU/ml (e.g. Polycheck or Euroline test). In addition, the test results are also presented in a semi-quantitative manner; in so-called classes from 0 (no specific antibodies) to 6 (antibody in very high concentration). The qualitative method only informs us of the presence of a specific antibody without giving information on its concentration. [11]

When testing allergen-specific IgE, we assess circulating antibodies in the blood; we do not determine the concentration of cell-bound IgE in tissues. This is the reason for the frequent false-negative results and therefore the lower sensitivity of serological tests than PTS. The authors also report that due to the possibility of local synthesis of IgE by organs, which has no significant effect on increasing its serum concentration, a patient may report symptoms of the disease despite negative diagnostic tests. [6]

As IgE testing based on the use of allergen extracts makes impossible to assess the occurrence of cross-reactivity, the possibility of allergen immunotherapy or the severity of a potential systemic reaction after contact with the allergen. The strong advantage of methods based on the use of allergen extracts are high availability, price and ease of interpretation. [8]

## Comparison of methods

In recent years, conventional methods for the determination of specific IgE have increasingly been supplemented by component-based diagnostics, detecting and determining antibody levels for individual allergen components or their molecules, which ensures a much more accurate diagnosis. In the assessment of sIgE, molecular diagnostics is carried out by single antibody determinations or by 'multiplex' method. In a situation with, for example, reactions to a number of different foods, it is more appropriate to use multiplex methods, such as the ALEX test, the FABER test or the ImmunoCAP ISAC test. The above methods allow the determination of sIgE concentrations for a large number of allergens (approximately 200) in a small amount of blood serum. [2]

Recent advances in *in vitro* allergy diagnostics have led to the development of both high-throughput multiplex laboratory platforms and rapid point-of-care (POC) assays, each addressing different clinical needs. Bernardini et al. provide a detailed overview of multiplex systems such as ISAC and ALEX<sup>2</sup>, while Nösslinger et al. evaluate the diagnostic performance of the Fast Check POC 20 Atopy test in comparison with a multiplex reference method. Multiplex systems, including ISAC and ALEX<sup>2</sup>, are based on array technologies that allow the simultaneous detection of IgE antibodies directed against a large number of allergenic components from a single serum sample. ISAC employs a microarray format with fluorescence-based detection, whereas ALEX<sup>2</sup> uses a macroarray platform with colorimetric readout. These technological differences influence analytical characteristics such as dynamic range, signal saturation, and data interpretation. A major advantage of multiplex systems is their extensive allergen coverage: ISAC includes 112 molecular allergen components, while ALEX<sup>2</sup> expands this approach by incorporating both molecular allergens and whole allergen extracts. This enables detailed molecular profiling and supports component-resolved diagnostics, particularly in patients with complex polysensitization patterns. Bernardini et al. emphasize that, despite overall good concordance, multiplex platforms are not analytically interchangeable due to differences in allergen composition, detection methods, and strategies for managing cross-reactive carbohydrate determinants (CCD). Consequently, results require careful interpretation by experienced clinicians, and multiplex testing is best positioned within specialized allergy care rather than as a first-line screening tool. In contrast, the Fast Check POC 20 Atopy test represents a fundamentally different diagnostic concept. As evaluated by Nösslinger et al., it is a lateral-flow immunochromatographic assay designed for rapid detection of IgE sensitization to a limited panel of 20 allergens or allergen mixtures. The test can be performed at the point of care, provides results within approximately 30 minutes, and requires minimal laboratory infrastructure. These features make it attractive for use in primary care settings or situations where rapid decision-making is needed. However, when compared with ALEX<sup>2</sup> as a reference method, Fast Check POC 20 Atopy demonstrated a marked trade-off between specificity and sensitivity. While specificity was high, indicating a low rate of false-positive results, overall sensitivity was limited, with a substantial proportion of sensitizations detected by multiplex testing not identified by the POC assay. Sensitivity was higher in patients with more severe allergic manifestations, suggesting that the

test may be more effective in detecting clinically overt atopy rather than low-level or asymptomatic sensitization. [12,13,14]

Table 1. Comparison of multiplex laboratory platforms and point-of-care allergy diagnostics.

Feature	ISAC / ALEX (Multiplex laboratory systems)	Fast Check POC 20 Atopy (Point-of-care test)
Reference article	Bernardini R et al., 2024	Nösslinger H et al., 2025
Diagnostic concept	Multiparametric / multiplex in-vitro diagnostics enabling simultaneous detection of IgE to multiple allergenic components	Rapid point-of-care immunoassay designed for quick screening of atopy
Technology	ISAC: microarray with fluorescence detection; ALEX <sup>2</sup> : macroarray with colorimetric detection	Lateral-flow immunochromatographic assay
Number of allergens/components	ISAC: 112 molecular allergens; ALEX <sup>2</sup> : 178 molecular allergens + 117 allergen extracts	20 predefined allergens or allergen mixes
Type of allergens	Predominantly molecular components (ALEX <sup>2</sup> additionally includes whole extracts)	Mainly allergen extracts / mixes
Sample type and handling	Serum sample analyzed in a specialized laboratory	Whole blood or serum; minimal preparation
Turnaround time	Several hours to days (laboratory workflow dependent)	Approximately 20–30 minutes
Quantitative output	Semi-quantitative (ISAC) or quantitative	Qualitative / semi-quantitative (positive/negative)

	(ALEX <sup>2</sup> ) IgE values	
Management of CCD interference	ISAC: use of recombinant components and CCD marker; ALEX <sup>2</sup> : CCD inhibition step	No specific CCD-blocking strategy reported
Diagnostic performance	High analytical sensitivity for a broad range of allergens; results not interchangeable between platforms	High specificity (~92%) but limited overall sensitivity (~43%) compared with ALEX <sup>2</sup>
Clinical role	Comprehensive molecular allergy diagnostics, particularly useful in complex polysensitization patterns	Screening and rule-out tool for atopy in primary care or resource-limited settings
Strengths	Broad allergen coverage; detailed IgE profiling; supports precision allergy diagnosis	Rapid results; easy to perform; suitable for decentralized testing
Limitations	Higher cost; need for expert interpretation; laboratory infrastructure required	Limited allergen panel; lower sensitivity; not suitable as a stand-alone diagnostic method
Recommended use according to authors	Advanced diagnostic evaluation and clinical decision support in specialized allergy care	Initial screening tool, complementary but not substitutive to laboratory diagnostics

The use of molecular methods has significantly improved the quality of allergy diagnostics. It makes it possible to distinguish primary sensitisation from cross-reactions, allows the selection of appropriate immunotherapy and in food allergy, it allows the determination of the risk of anaphylaxis [15,16]. This is important in the patient's following of a diet that excludes allergenic products. The result of the test in which extracts are used for assessing the allergy, determines only the presence of antibodies to a specific food, resulting in the patient being advised to eliminate this protein from the diet. Given that these proteins are thermolabile or digestible through the digestive tract, it is not necessary to exclude them completely from the diet, but the patient must remember to subject the product to heat treatment, for example.

However, many other allergens, e.g. mainly peanut or fish allergens, are thermostable. Component methods provide the answer to such questions. It is also important to carry out further diagnostics on the patient, e.g. to perform a challenge test.

### CCDs

It is also worth mentioning the commonly occurring bicarbonate residues in the body – CCDs (cross-reactive carbohydrate determinants). Their presence can affect up to 30 % of patients. CCDs can induce the formation of IgE antibodies and react with them, but they do not induce mast cell degranulation. [17] Due to their high structural similarity to many different allergens, they are the cause of numerous cross-reactions and, consequently, false-positive results of *in vitro* tests. Viewed practically, the detection of anti-CCD antibodies can mimic a state of polyvalent allergy (polysensitisation) particularly in situations of allergy to pollen, hymenoptera insect venom, latex, and foods of plant origin. [14] The use of a CCD inhibitor or recombinant allergens devoid of carbohydrate determinants avoids false positives. This is a unique feature of the ALEX test. It is worth noting that not all molecular diagnostic platforms routinely use CCD inhibitors or offer only recombinant allergens without CCD. [15, 18, 19]

The occurrence of cross-reactions greatly complicates the diagnosis and correct treatment of the patient. The similar chemical structure of the individual allergen components favours the reaction of specific antibodies with many different proteins. Examples include profilins, a group of proteins that show a high degree of cross-reactivity between species (e.g. birch-apple).

When diagnosing a patient, you should keep in mind the ignorance of all molecules forming the antigen or the inability to use them for testing. Allergy diagnostics should therefore include not only component testing, but also extracts. This increases the likelihood of correctly diagnosing a patient, avoiding the omission of individuals sensitised to molecules not available for component diagnosis. [20]

The test result obtained for an extract is not always consistent with the result for an individual molecule. The result obtained for a test extract can be negative in a situation where the patient has specific E antibodies for a particular allergenic molecule in the body. This may be due to the low content of a particular molecule, its complete absence from the allergen extract, or its destruction during the preparation of the extract for testing. [21, 22].

Table 2. The following table made on the basis of an article by Samolinski et al. gives examples of the reasons for the differences in the results of molecular tests and conventional methods [8].

Extract	Molecule	Explanation
negative	negative	Exclusion of IgE-dependent allergy
positive	negative	Presence of anti-CCD antibodies in the patient's blood  Presence of molecules not available for examination in a given test
negative	positive	Lower sensitivity of test based on extract alone  Insufficient amount of molecule in the extract

### Material and Methods

A literature review was conducted regarding methods of allergy diagnostics. Both traditional *in vivo* techniques, such as skin tests (prick, intradermal, epidermal) and provocation tests, were included [2]. Available *in vitro* methods were also analyzed, including the assessment of allergen-specific IgE in serum and the determination of total IgE concentration [7]. The latest reports on rapid point-of-care (POC) immunoassays and modern molecular techniques, including component resolution diagnostics and multiparameter tests such as ALEX, FABER, and ImmunoCAP ISAC, were also included. [5,12,13,14,23,25]. Literature was searched in PubMed and Google Scholar databases, as well as in specialist textbooks [22,23].

### Discussion

Classical *in vivo* tests have been the cornerstone of allergy diagnostics for many years; however, their limitations include subjective interpretation of results and the risk of systemic reactions [2,7]. Serological *in vitro* methods allow for safe and reproducible assessment of allergen-specific IgE levels, although their sensitivity is often lower compared to skin prick tests [2,5].

In recent years, molecular diagnostics have significantly expanded clinical possibilities, enabling differentiation between primary allergies and cross-reactions, qualification for specific immunotherapy, and assessment of anaphylaxis risk [6,20,25]. In the context of extended diagnostic workup, multiplex assays - enabling the simultaneous assessment of IgE reactivity to multiple allergenic components - have garnered increasing interest. However, the decision to employ such tests should be made on an individual basis, depending on the specific clinical scenario and the recommendations of a specialist. As pediatric studies have shown, multiplex assays may detect sensitizations with very low clinical relevance, which - as noted by

Sonneveld et al. - can lead to overinterpretation of results and the implementation of unnecessary, and in some cases even “absurd,” elimination diets. For this reason, both Casas et al. and other authors emphasize that *in vitro* test results, especially those generated by multiplex platforms, should be interpreted with caution and always in the context of symptoms, allergy history, and, when necessary, provocation testing. [3,4].

**Summary**

Since the introduction of molecular methods into diagnostics, we can increasingly see them displacing the conventional determination of sIgE by extracts. However, the 'top-down' diagnostic model shows that conventional methods of determining sIgE antibodies using extracts should be used in the second stage of diagnostics, after the patient interview, which is the primary diagnostic tool. At the point of incomplete diagnosis, the third step should be the use of molecular technology [8,10,23].

There is also a 'bottom-up' approach, which recommends starting the diagnostics directly with molecular methods in order to significantly shorten the diagnostic pathway.

A number of tests are currently available both using extracts and molecular techniques for testing. It is important to know the specifics of the tests in order to reliably select the right test for the patient's needs. Based on scientific evidence and accumulated experience in daily clinical practice, it is clear that the results of different commercial *in vitro* tests for allergy diagnosis are not comparable. [5, 24-27]

It is important to remember that tests for the diagnostics of allergy should have high specificity and sensitivity, have sufficient scientific evidence to support their clinical utility, cover a broad spectrum of allergens, both in extract and molecule form, and be cost-effective.

Both conventional methods and molecular techniques should go hand in hand with a thorough clinical history and analysis of the patient's symptoms. Despite the many differences in allergy diagnosis tests using extracts or molecules, it is reasonable to use them simultaneously in order to be able to treat the patient optimally.

Table 3. Comparison of conventional methods and molecular diagnostics for allergy evaluation.

	Conventional methods	Molecular methods
Level of analysis	extracts	Mainly allergenic molecules/components; some platforms may also contain extracts
Application	Diagnosis of IgE-mediated allergy	Diagnosis of IgE-mediated allergy
Cross reactions	No possibility of assessing cross reactions	Differentiating cross reactions from primary allergy

Immunotherapy	Problems in multi-allergy situations.	Ability to accurately qualify for immunotherapy
Anaphylaxis	Inability to assess the risk of anaphylaxis	Possible anaphylaxis risk assessment
Cost	Lower cost of performing the test	Higher cost of performing the test
Example	Polycheck – food panel IV Polycheck - inhalation panel IV The classic EUROLINE test FastCheckPO C 20 Atopy	ALEX test Faber test ImmunoCAP ISAC Polycheck gluten-milk

**Conclusions**

Cross-sectional studies based on medical record analyses indicate that the use of skin-prick testing (SPT) is a valuable first-line approach in the evaluation of individuals with suspected allergy to airborne allergens [3]. Additionally, the allergen extracts used in SPT contain proteins that have not yet been characterized, and we currently lack the ability to assess their IgE reactivity in patient serum. For example, recent proteomic analyses have identified up to 3,000 newly detected proteins for each pollen taxon, which may contribute to their variable allergenic potential [4]. A diagnostic challenge may arise in cases where IgE reactivity and allergic inflammation are confined to the local nasal mucosa, without detectable IgE either in skin-prick testing or in serum [4].

Accurate diagnosis of IgE-mediated allergic diseases requires an integrated, stepwise approach that combines a thorough clinical evaluation with the appropriate selection of diagnostic tools, including both conventional extract-based tests and advanced molecular techniques. While extract-based assays remain widely used due to their availability, lower cost, and ease of interpretation, they are limited by cross-reactivity, batch-to-batch variability, and their inability to identify specific sensitizations at the molecular level.

In contrast, molecular allergy diagnostics - particularly component-resolved diagnostics - offer significant clinical advantages. These methods enable precise identification of sensitizing allergen components, differentiation between primary sensitization and cross-reactivity, and assessment of the risk of severe allergic reactions such as anaphylaxis. They also provide essential guidance for personalized immunotherapy and dietary management. Multiplex platforms like ALEX, FABER, and ImmunoCAP ISAC allow simultaneous testing for a broad range of allergen components from a small serum volume, which is particularly beneficial in polysensitized patients or complex clinical scenarios.

However, molecular tests are not without limitations. One of the key challenges in *in vitro* diagnostics is the presence of cross-reactive carbohydrate determinants (CCDs), which can lead to false-positive results. The use of CCD inhibitors or recombinant allergens devoid of carbohydrate moieties can significantly improve diagnostic specificity, representing a clear advantage of certain molecular platforms.

Importantly, no single diagnostic method is sufficient on its own. Conventional and molecular approaches should be regarded as complementary, not mutually exclusive, with the choice of testing strategy tailored to the individual patient's clinical history, symptomatology, and diagnostic context. A detailed clinical interview remains the cornerstone of allergy diagnosis, and laboratory tests should be used to support, not replace, clinical judgment.

Finally, the lack of standardization among commercially available *in vitro* assays can lead to inconsistencies in test results. Therefore, a thorough understanding of the characteristics, strengths, and limitations of each diagnostic method is essential for making informed and clinically relevant decisions in allergy management.

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